J. Perinat. Med.
17 (1989) 113

# Inhibitors and activators of fibrinolysis during and after childbirth in maternal and cord blood

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## 1 Introduction

During pregnancy physiological alterations occur in the coagulation and fibrinolytic systems. Biezinski et al. reported in 1958 [4] that plasma fibrinolytic activity, measured in euglogulin precipitates, is decreased in pregnant women. These alterations may play an important role in placental haemostasis.

Several studies have shown a rapid rise of the plasma fibrinolytic activity shortly after childbirth and separation of the placenta [13, 14]. During the process of placental separation a blood flow of 500–800 mls/min to the placental bed has to be staunched. The activation of the coagulation in the placental bed has to be localized to the uterus to prevent intravascular clotting. This is achieved by the inhibitors of coagulation and the fibrinolytic system.

In the newborn infant an increased fibrinolytic activity has been reported [3].

## 2 Patients and methods

### 2.1 Subjects

Twenty-six healthy pregnant women (age range 16–35 years) at term with no evidence of preeclamsia, eclamsia or overt thromboembolic disease were studied. None of these women had a history of thromboembolic disease.

Of the twenty-six subjects thirteen pregnant women were primigravidae. Twenty-two patients were studied during normal childbirth, four patients during elective caesarean section. Full and informed consent was given by each patient for specimens of blood to be taken with a minimum of venous occlusion from an ante-cubital vein in the second and third stage of labour, and at 48 and 72 hours after delivery. Blood was also taken from the umbilical vein on the maternal side immediately following childbirth, after clamping the cord, and befor delivery of the placenta.

### 2.2 Fibrinolytic assays

5 ml of blood was taken directly into plastic syringes containing sodium citrate as an anticoagulant with a minimum of venous occlusion. For determination of functional t-PA activity 2 ml of blood was immediately mixed with 1 ml of 10%

**Curriculum vitae**

INGO BERNARD RUNNEBAUM *was born in Köln, West-Germany, in 1960. From 1980 to 1982 he studied chemistry and philosophy at the University of Heidelberg and from 1982 to 1988 medicine at the Universities of Mainz, Berlin and Munich. His clinical training included operative gynecology in Buenos Aires, Argentina, in 1985; obstetrics in Dublin, Ireland, in 1987; plastic and reconstructive surgery in Bangkok, Thailand, in 1988. His main fields of interest include molecular biology and the immunology of fertility and pregnancy. Since January 1989 he has started a research project at the Salk Institute in San Diego, USA.*

acetate buffer, centrifuged and further acidified with 20% acetic acid to assure a pH of 4.0–4.1. All samples were snap-frozen in liquid nitrogen, stored at −80 °C and assayed within four weeks.

t-PA antigen was determined using a sensitive enzyme linked immunosorbent assay (ELISA) utilizing the double antibody principle [17]. This assay measures not only free t-PA but also inhibitor-ecomplexed t-PA. The t-PA functional activity was measured using an enzymatic assay involving a three-stage reaction: t-PA is stimulated by soluble des-AA fibrin, glu-plasminogen is converted to plasmin by catalysation of stimulated t-PA, the substrate D-But-CHT-Lys-pNA is then hydrolysed to free pNA by plasmin [18]. In this assay all free t-PA inhibitors present were destroyed by acidification of the sample. Thus the total t-PA activity could be measured.

For determination of the t-PA inhibitor an indirect enzymatic assay was performed in two stages [5]. In stage 1 t-PA reacts with the free PAI present in the specimen. In stage 2 the residual t-PA activity is measured by using the t-PA activity assay. The PAI content is then determined as the difference between the amount of t-PA added and the amount of t-PA found.

### 2.3 Statistical analysis

Data were analysed using the Mann-Whitney-Wilcoxon test and the paired t-test. Time-series cross-correlation analysis was applied to the data. Mean values are reported along with the standard error of the mean.

## 3 Results

### 3.1 t-PA Antigen

t-PA antigen levels in maternal plasma in the second stage of labour were found to be three fold higher than in the plasma of non-pregnant women (8.66 ng/ml, S. D. 4.75 ng/ml). During late labour from second to third stage t-PA antigen increased significantly ($p < 0.05$, Mann-Whitney-Wilcoxon) to 11.96 ng/ml (S. D. 4.61 ng/ml). A marked decrease of t-PA levels ($p < 0.001$) was observed between the third stage of labour and 48 and 72 hours after delivery (figure 1).

The mean t-PA antigen values in the cord blood of the newborn (9.18 ng/ml, S. D. 6.94 ng/ml) were found to be in the same range as the mothers' levels in the second stage of labour. A significant difference ($p < 0.01$) to maternal t-PA antigen levels in the early puerperium was observed (figure 2).

No significant differences in the t-PA antigen levels were found when comparing the primiparous patients with the multiparous patients or the patients who delivered by caesarean section with the patients with spontaneous delivery.

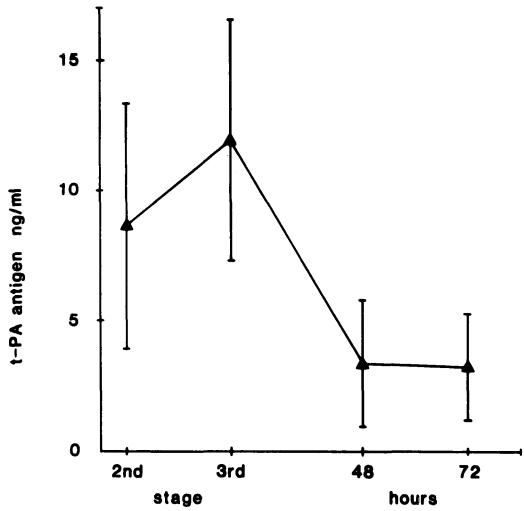


**Figure 1.** Plasmat-PA antigen levels during and after normal childbirth. The results are expressed as the mean ± the standard deviation.

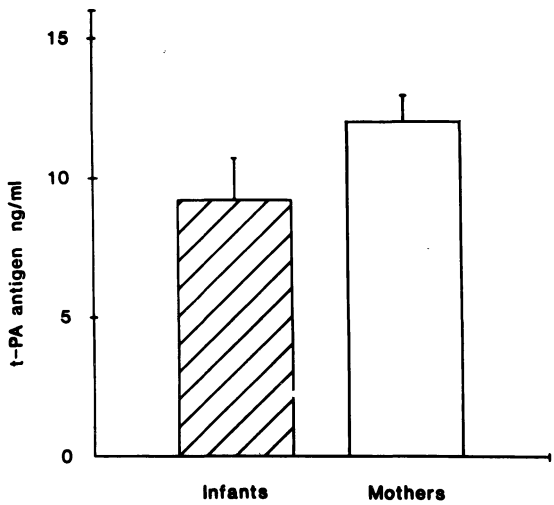


**Figure 2.** Comparison of t-PA antigen levels in cord blood and maternal blood before delivery of the placenta. The results are expressed as the mean ± the standard error of the mean.

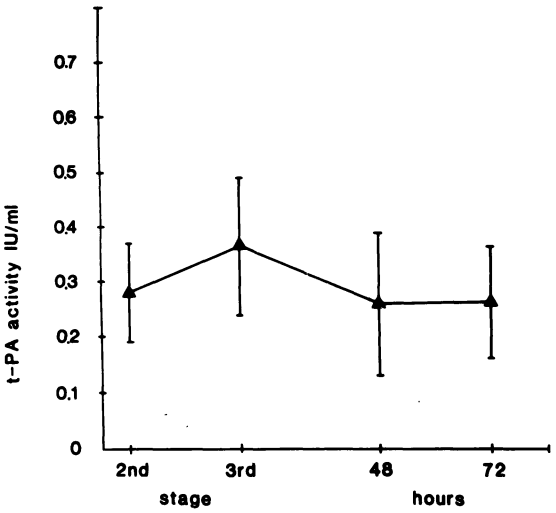


**Figure 3.** Plasmat-PA activity levels during and after normal childbirth. The results are expressed as the mean ± the standard deviation.

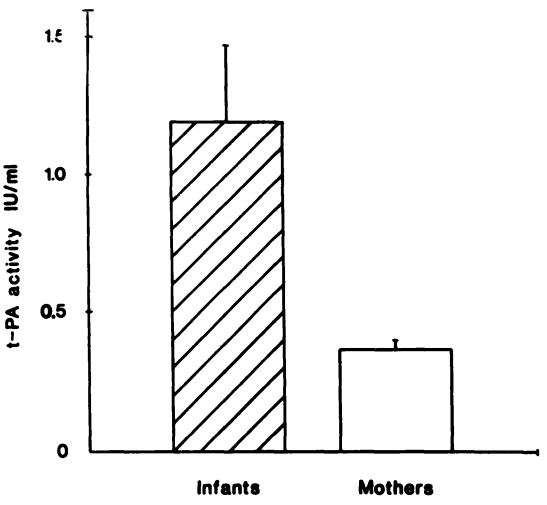


**Figure 4.** Comparison of t-PA activity levels in cord blood and maternal blood before delivery of the placenta. The results are expressed as the man ± the standard error of the mean.

### 3.2 t-PA Activity

Figure 3 shows the total t-PA activity during late labour and early puerperium. The trend obtained was similar to that found when measuring the t-PA antigen levels. The changes were statistically significant when comparing the second (0.278 IU/ml, S. D. 0.093 IU/ml) with the third stage (0.365 IU/ml, S. D. 0.124 IU/ml) of labour ($p < 0.05$, Mann-Whitney-Wilcoxon) and the third stage of labour with 48 hours (0.258 IU/ml, S. D. 0.127 IU/ml) and 72 hours (0.263 IU/ml, S. D. 0.100 IU/ml) after delivery ($p < 0.05$). The t-PA activity showed an increase of more than 30% between second and third stage of labour. At 48 and 72 hours after childbirth the values had returned almost to those of the second stage of labour.

The t-PA activity found in the umbilical cord plasma was high (1.190 IU/ml, S. D. 1.108 IU/ml) when compared to the maternal plasma during both stages of labour ($p < 0.003$). The difference was also highly significant when comparing the cord values with those of the first three days post partum ($p < 0.001$). The relation between the cord t-PA activity and the plasma t-PA activity in the third stage of labour is shown in figure 4.

The t-PA activity levels in the newborn cord blood after ceasarean section were found to be significantly lower than in the cord blood after spontaneous uncomplicated delivery ($p < 0.03$). No significant difference was found between primiparae and multiparae.

### 3.3 t-PA Inhibitor

The total inhibitory activity of PAI in plasma was found to be five to six times higher in the 2nd stage of labour (22.84 IU/ml, S. D. 6.21 IU/ml) than in normal human plasma and showed a slight but nonsignificant decrease in the third stage of labour (22.43 IU/ml, S. D. 5.50 IU/ml). At 48 hours after delivery (9.71 IU/ml, S. D. 4.31 IU/ml) a decline of more than 50% ($p < 0.001$) in PAI activity was observed. 72 hours after delivery (9.61 IU/ml, S. D. 4.74 IU/ml) the values still remained at this low level. The difference was not significant when compared with the values at 48 hours post delivery (figure 5).

PAI plasma levels found in the cord blood were approximately one third of the maternal plasma levels (7.06 IU/ml, S. D. 6.08 IU/ml) during the third stage of labour ($p < 0.001$) (figure 6). Also the 48 hours PAI plasma levels of the mother were significantly higher ($p < 0.05$) than the cord PAI levels.

No difference was found between primiparae and multiparae or patients with vaginal delivery and patients who delivered by caesarean section.

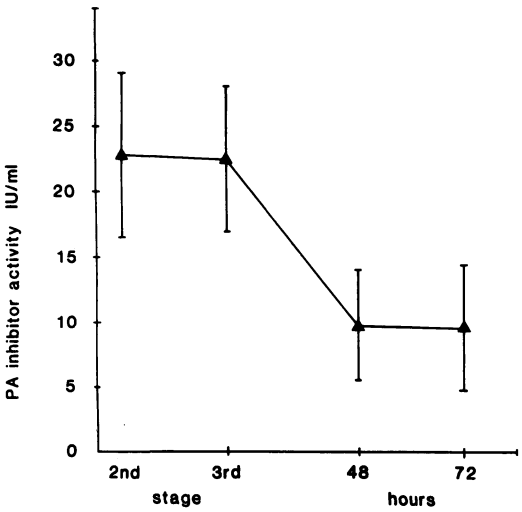


**Figure 5.** Plasma PA inhibitor activity levels during and after normal childbirth. The results are expressed as the mean ± the standard deviation.

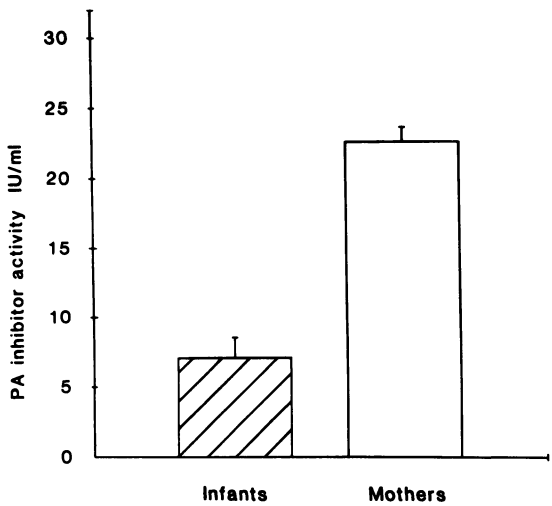


**Figure 6.** Comparison of PA inhibitor activity levels in cord blood and maternal blood before delivery of the placenta. The results are expressed as the mean ± the standard error of the mean.

### 3.4 Time series cross-correlation

When t-PA antigen values (figure 1) were cross-correlated with t-PA inhibitor values (figure 5) a cross-correlation coefficient of 0.94 was obtained.

A cross-correlation coefficient value of 0.89 was obtained when the t-PA antigen (figure 1) time series was cross-correlated with the t-PA activity (figure 3) time series.

**Table.** Significance values of t-PA antigen and PA inhibitor activity in newborn cord plasma and in maternal plasma during labour and early puerperium. (Mann-Whitney-Wilcoxon-Test)

| | t-PA Antigen | | | | |
|---|---|---|---|---|---|
| | 2nd stage | 3rd stage | 48 hours | 72 hours | cord |
| 2nd stage | – | $p < 0.05$ | $p < 0.001$ | $p < 0.001$ | n. s. |
| 3rd stage | n. s. | – | $p < 0.001$ | $p < 0.001$ | n. s. |
| 48 hours | $p < 0.001$ | $p < 0.001$ | – | n. s. | $p < 0.01$ |
| 72 hours | $p < 0.001$ | $p < 0.001$ | n. s. | – | $p < 0.01$ |
| cord | $p < 0.001$ | $p < 0.001$ | $p < 0.05$ | n. s. | – |

t-PA Inhibitor

## 4 Discussion

Previous studies in coagulation and fibrinolysis during uncomplicated pregnancy and normal childbirth have indicated profound changes in the haemostatic system towards hypercoagulability [11, 9]. Both blood coabulation and fibrinolytic parameters return to normal within the first three weeks of puerperium [7]. The overall fibrinolytic activity of newborn cord blood has been reported to be on a higher level than that of the mother at term [8, 10].

Our data confirm that marked changes in the levels of t-PA antigen, activity and inhibitor take place during delivery and in the early puerperium. t-PA antigen levels have been reported to be increased in the second half of pregnancy [13]. Previous studies [13, 14] have revealed a further rise in t-PA antigen shortly after delivery of the placenta. Our results show that the t-PA antigen levels in maternal plasma increase immediately following childbirth, before delivery of the placenta. This rise may be explained by a t-PA release from the vessel walls in the placental bed during the process of placental separation.

Oxytocin and stress hormones such as epinephrine have been shown to be capable of freeing plasminogen activators [6]. Strong contractions of uterine myometrium during labour and in particular in the late second stage can cause a t-PA release from the vascular endothelium.

Previous studies of plasma t-PA activity before and after childbirth revealed conflicting results more likely due to the different assays used [13, 14]. We found a significant increase of t-PA activity between the second and the third stage of labour; to our knowledge none of the previous studies had investigated this period immediately after delivery of the baby.

The increase corresponds to the trend of the t-PA antigen levels found in maternal plasma. Mackinnon et al. [14] showed that the t-PA antigen decreases to near normal values three to five days after delivery. Our findings are similar, at 48 hours after childbirth a steep decrease to near normal values had already occurred. The values at 72 hours remained in the same range. The t-PA activity pattern in maternal plasma follows the same trend as the t-PA antigen pattern although the significant changes are less striking.

During the last few years several studies on t-PA inhibitors have reported the existence of at least three distinct plasminogen activator inhibitors: PAI 1, the so called fast acting inhibitor, purified from endothelial cells [16], PAI 2, the placental type inhibitor found in trophoblastic endothelium [1, 2] and protease nexin [19]. To determine the overall fibrinolytic status of the mother the present study investigated the relation between the total inhibitory activity andthe activator activity without discriminating between the different types of plasminogen activator inhibitors. In recent studies high levels of PAI activity were found in the second stage of labour, the reported PAI activity values measured shortly after delivery of the placenta showed a significant decline [13, 14]. Our data confirm these second tate PAI activity findings. However we found that the PAI activity plasma levels in the third stage before placental delivery still remained at this high level. At 48 hours after delivery a striking decrease in the overall PAI activity was fond while the measurement at 72 hours showed no further change. These results are possibly due to the separation of the placenta which is known to be a rich source of fibrinolytic inhibitors [12]. With placental separation an important site of inhibitor production in pregnancy ceases resulting in the significant decrease in inhibitory activity found in maternal plasma after placental separation.

Previous studies [15] have compared the fibrinolytic system in the newborn cord blood with that in the mothers' plasma at term and have found profound differences between both groups. In the present study blood was taken simultaneously from the cord and the mother before placental delivery. Thus the changes in the fibrinolytic activity could be investigated just as the placenta was separating. We found higher maternal levels of t-PA antigen in the third stage of labour than in the cord while the t-PA activity levels were lower in the mother when compared with the cord blood. This can be explained by the high concentration of PAI activity present in the mothers' blood at the third stage of labour. The PAI activity in the cord was found to be very low so the t-PA antigen will bind to the inhibitor in a reduced quantity. Therefore high t-PA activity levels were found in the infant cord blood. This high level of fibrinolytic activity will serve as a pretective mechanism to prevent fibrin formation in the baby during and after delivery [10]. The total inhibitory activity of PAI was low in the cord before separation of the placenta suggesting that the endothelial cells in the umbilical cord are not involved in PAI production. PAI seems to be selectively released into the maternal circulation. The absence of fibrinolytic activity found around cytotrophoblastic cells was considered to be due to a high level of inhibitor in these cells [20]. To clarify the origin of present inhibitors it would be interesting to investigate the type of PAI found in the cord and in cytotrophoblasts by immunological means.

**Abstract**

During normal childbirth profound changes in the fibrinolytic system take place. Tissue plasminogen activator (t-PA), the antigen and its biological activity and the activity of plasminogen activator inhibitor (PAI) were measured in twenty-two healthy women during and shortly after spontaneous delivery (2nd stage and 3rd stage of labour, 48 and 72 hours post partum). Significant increases of plasma t-PA antigen and activity occurred during childbirth and before delivery of the placenta, while the inhibitor remained unchanged. After delivery the PA inhibitor and t-PA antigen showed a steep decline. The activity of t-PA remained largely unchanged during labour and after delivery.

The comparison between the activity levels of PAI in infant cord blood and in maternal peripheral blood, taken simultaneously during the process of placental separation, showed significantly higher PAI activity in the mother. In contrast the levels of t-PA activity were found to be significantly higher in cord blood.

Our results demonstrate that during the process of childbirth and separation of the placenta distinct alterations in the fibrinolytic system occur most likely due to placental effects.

**Keywords:** Fibrinolysis, pregnancy, puerperium, tissue plasminogen activator, tissue plasminogen activator inhibitor.

**Zusammenfassung**

**Inhibitoren und Aktivatoren der Fibrinolyse im mütterlichen Blut und im Nabelschnurblut während und nach der Geburt**

Während der normalen Entbindung finden umfassende Veränderungen im fibrinolytischen System statt. Bei 22 gesunden Frauen wurden während und kurz nach einer Spontangeburt (2. und 3. Phase der Wehentätigkeit, 48 und 72 h post partum) der zellgebundene Plasminogen-Aktivator (t-PA), einmal als Antigen selbst und zum anderen seine biologische Aktivität, sowie die Aktivität des Inhibitors vom Plasminogen-Aktivator (PAI) gemessen. Während der Entbindung und vor der Geburt der Plazenta kommt es zu einem signifikanten Anstieg des t-PA-Antigen und seiner Aktivität im Plasma, während der Inhibitor unverändert bleibt. Nach der Entbindung fallen der PAI und das t-PA-Antigen steil ab. Die Aktivität des t-PA bleibt während und nach der Geburt weitgehend konstant.

Der Vergleich zwischen den PAI-Aktivitätsspiegeln im Nabelschnurblut und im maternalen peripheren Blut (die Proben wurden simultan während der Abnabelung entnommen), zeigte eine deutlich höhere PAI-Aktivität bei der Mutter. Im Gegensatz dazu war die t-PA-Aktivität im Nabelschnurblut signifikant höher.

Unsere Ergebnisse zeigen, daß während der Geburt und nach Ablösung der Plazenta deutliche Veränderungen im fibrinolytischen System stattfinden, die am ehesten von der Plazenta ausgehen.

**Schlüsselwörter:** Fibrinolyse, Schwangerschaft, Wochenbett, zellgebundener Plasminogen-Aktivator und sein Inhibitor.

**Résumé**

**Inhibiteurs et activateurs de la fibrinolyse pendant et après la naissance dans le sang maternel et au sang du cordon**

Des modifications importantes du système fibrinolytique surviennent au cours de l'accouchement normal. Chez 22 femmes en bonne santé on a dosé pendant et peu parès la délivrance naturelle (2ème et 3ème parties du travail, 48 et 72 heures après) l'activateur du plasminogène tissulaire (t-PA), l'antigène et son activité biologique ainsi que l'activité de l'inhibiteur de l'activateur du plasminogène (PAI). Pendant la naissance et avant la délivrance, il existe une augmentation significative de l'antigène t. PA plasmatique et de son activité, alors que l'inhibiteur demeure inchangé. Après la délivrance, il existe une diminution de l'inhibiteur PA et de l'antigène t. PA. L'activité du t. PA ne se modifie pas au cours tu travail. et après la délivrance.

La comparaison entre les taux d'activité du PAI au sang du cordon et dans le sang périphérique maternel, recueillis simultanément au cours du décollement placentaire montre une activité PAI significativement plus élevée chez la mère. A l'inverse les taux d'activité t. PA sojt significativement plus élevés au snag du cordon.

Nos résultats démontrent qu'au cours de la naussance et du décollement placentaire surviennent des perturbations du système fibrinolytique secondaires très vraissemblablement à des effets placentaires.

**Mots-clés:** Activateur tissulaire du plasminogène, fibrinolyse, grossesse, inhibiteur de l'activateur tissulaire du plasminogène, post-partum.

**References**


[1] ÅSTEDT B, I LECANDER, T BRODIN, A LUNDBLAD, K LÖW: Purification of a specific placental plasminogen activator inhibitor by monoclonal antibody and its complex formation with plasminogen activator. Thromb Haemost 53 (1985) 122

[2] ÅSTEDT B, I HAEGERSTRAND, I LECANDER: Cellular localisation in placenta of placental type plasminogen activator inhibitor. Thromb Haemost 56 (1986) 63

[3] BELLER FK, GW DOUGLAS, MD EPSTEIN: The fibrinolytic enzyme system in the newborn. Am J Obstet Gynaecol 96 (1966) 977

[4] BIEZINSKI JJ, HC MOORE: Fibrinolysis in pregnancy. J Clin Pathol 11 (1958) 306

[5] CHMIELEWSKA J, M RANBY: Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. Thromb Res 31 (1983) 427

[6] COLLEN D: On the regulation and control of fibrinolysis. Thromb Haemost 43 (1980) 77

[7] DAHLMANN TH, M HELLGREN, M BLOMBÄCK: Changes in blood coagulation and fibrinolysis in the normal puerperium. Gynaecol Obstet Invest 20 (1985) 37

[8] EKELUND H, U HEDNER, IM NILSSON: Fibrinolysis in newborns. Acta Paediat Scand 59 (1970) 33

[9] GORE M, S ELDON, KF TROFATTER, S-J SOONG, SV PIZZO: Pregnancy-induced changes in the fibrinolytic balance: evidence for defective release of tissue plasminogen activator and increased levels of the fast acting tissue plasminogen activator inhibitor. Am J Obstet Gynaecol 156 (19879 674

[10] HATHAWAY WE, J BONNAR: Haemostatic Disorders of the pregnant woman and the newborn infant. Elsevier, New York 1987

[11] HELLGREN M, M BLOMBÄCK: Studies in blood coagulation and fibrinolysis in pregnancy during delivery and in the puerperium. Gynaecol Obstet Invest 12 (1981) 141

[12] KAWANO T, K MORIMOTO, Y UEMURA: Urokinase inhibitor in human placenta. Nature 217 (1968) 253

[13] KRUITHOF EKO, C TRAN-THANG, A GUDINCHET, J HAUERT, G NICOLOSO, C GENTON, H WELTI, F BACHMANN: Fibrinolysis in pregnancy: a study of plasminogen activator inhibitors. Blood 69 (1987) 460

[14] MACKINNON S, ID WALKER, JF DAVIDSON, JJ WALKER: Plasma fibrinolysis during and after normal childbirth. Br J Haematol 65 (1987) 339

[15] MACKINNON S, ID WALKER, JF DAVIDSON, JJ WALKER: Fibrinolytic activity in the healthy newborn infant at term. Fibrinolysis 1 (1987) 117

[16] MOURIK JA VAN, DA LAWRENCE, DJ LOSKUTOFF: Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. J Biol Chem 259 (1984) 14914

[17] RANBY M, N BERGSDORF, T NILSSON, G MELLBRING, B WINBLAD, G BUCHT: Age dependence of tissue plasminogen activator concentrations in plasma as studied by an improved enzyme-linked immunosorbent assay. Clin Chem 32 (1986) 2160

[18] RANBY M, B NORMAN, P WALLEN: A sensitive assay for tissue plasminogen activator. Thromb Res 27 (1982) 743

[19] SCOTT RW ET AL.: Protease nexin. J Biol Chem 260 (1985) 7029

[20] SHEPPARD BL, J BONNAR: Fibrinolysis in decidual spiral arteries in late pregnancy. Thromb Haemost 39 (1978) 751


Received August 30, 1988. Accepted January 24, 1989.

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